QUESTIONS FOR HEARING EXPERTS ON FIELD RESIDUES STUDIES

General

1. Is there a protocol under development providing guidance on how to design a study/studies to collect information on pesticide residue levels for pollen and nectar? Yes, per the Field Residue Writing Group of the Semi-field/Full Field Working Group of the ICPPR Bee Protection Group.
2. In your opinion/experience, the semi-field or the open field design is the more appropriate? Why? This depends on the objectives of the study. If the objective is to track the fate (dissipation) of the pesticide from the plant nectar/pollen to the bee collected nectar/pollen to the hive products (honey, bee bread) under worse case foraging conditions, then a semi-field/tunnel study is desirable. Furthermore, some crops can only be realistically sampled using bees (in tents). Generally, however, an open (full field) design is preferable for logistical efficiency, better environmental realism (no interference from tunnels/tents), and better representation of typical agronomical practices (irrigation, etc).
3. Does this protocol (or any) include a reasoning for the number of sites and number of samples to be taken in order to provide a reasonable representative sampling regime for a certain pesticide use in a certain scale (i.e. considering spatial and temporal scale factors)? In my opinion, no current guidance for conducting bee relevant residue studies provides a precise reasoning for the recommended number of sites (e.g., USEPA = 3 minimum; EFSA = 5 minimum; CDPR ~ 9 minimum). For the neonicotinoids, site-to-site variability in residue concentrations (measured on the same crop, at the same rate and timing) can vary up to 100X (typically 10X). I believe an analysis of spatial variability in residue data is needed to better justify the number of sites needed in bee-residue studies.

   Given the limitations of the current residue database for pollen and nectar, I believe an analysis of residue trial data used to support human health risk assessment might provide valuable insights. At USEPA, such "magnitude of residue" trials are conducted on a large number of sites (12+ sites/crop) depending on the use pattern. USEPA is planning on exploring such an analysis in the near future.
4. Do you think that such a protocol should be different for different pesticide application types such as spray application(s) during the flowering, spray application(s) before the flowering, seed treatment and or other types of application(s) during or before the flowering? Yes! We know for the neonicotinoids, the residue profile over time differs greatly among application methods (foliar spray, soil, seed treatment) in addition to the timing of application (pre/during bloom; post bloom). These differences WILL impact the occurrence of the "peak" residues and the overall temporal variability. This in turn will

impact the timing and number of samples needed to be taken. Protocol differences will also likely depend on the systemicity of the chemical, timing of application and the persistence of the pesticide on/in foliage and soil.

5. How can it be ensured that the peak concentration is captured? For foliar spray applications at bloom, this is readily done by ensuring that sampling begins within 1 day after application (and bloom) occurs. For foliar applications made prior to bloom, peak residues would likely be captured during the first days of bloom. For soil applications, capturing "peak" residues is more difficult because of the competing effects of factors like the rate of chemical uptake from soil, rate of crop growth, formation rates of pollen and nectar, and degradation rates of the chemical in soil and plant tissues. This is especially difficult for indeterminate blooming crops (cotton, cucurbits) which have long bloom periods. in these cases, frequent sampling during the entire bloom periods is required. For seed treatments, sampling early in the bloom period will likely capture peak residues, however exceptions can occur (e.g., imidacloprid in seed treated corn pollen increased over the bloom period presumably to desiccation of pollen).
6. Do you consider any criteria to select the sites where to conduct field residues studies? Do you think it would be possible to define "representative sites" (in terms of environmental factors) where to conduct field residue studies? The only criterion currently used is that the site(s) be representative of the dominant locations where the crop is grown. In terms of environmental factors as criteria, I believe these will be dependent on the physicochemical properties of the pesticide and to some extent, how it is used. For example, if a persistent, systemic pesticide preferentially partitions to organic carbon and is soil applied, then criteria may be established based on soil properties (e.g., % organic carbon, % sand/silt/clay).

   That being said, agronomic practices (irrigation) and other factors could offset such 'environmental' criteria, especially for systemic pesticides. Ultimately, plant uptake and translocation models are needed to address this problem, because the expression of residues in pollen and nectar is not simply a function of a pesticide's bioavailability in soil, for example. Rather, such residues depend on the mechanism of translocation (apoplastic, xylem, phloem mediated transport), the timing of application relative to production of pollen & nectaries, the growth rate of the plant, transpiration, among other factors.

   In terms of defining "representative sites", I think it might be possible to do this from an environmental perspective, perhaps building upon the ecoregion approach used by USEPA and others for Terrestrial Field Dissipation studies. This may be more applicable to

foliar applications of non-systemic chemicals. Again, however, such environmental factors for defining representative could be confounded by biological and agronomic factors, particularly for systemic compounds.

7. If a seed-treated crop can be sown in autumn as well as in spring (e.g. wheat), are there indications whether the autumn or spring variant will result in the highest residues in nectar and pollen? Not to my knowledge. While a considerable amount of residue data have been generated for seed treatment applications of pesticides, the confounding influence of site-to-site differences may outweigh the influence of season on residues (particularly since residues from seed treatments tend to be very low---based on the neonicotinoids). I believe to properly address this question, one would need to have a study conducted at the same site, crop, application rate but with applications at the different seasons.

Study design

8. Do you recommend a minimum size for field plot in the semi-field and open field trials? This question seems to be getting into the effects side of semi-field/full field studies. Semi-field (tunnel) studies have defined plot sizes in OECD guidelines and additional recommendations from ICPPR (larger tunnels are generally better). For full field studies, the plot size has not been precisely defined to my knowledge. This question was posed to the USEPA FIFRA Scientific Advisory Board in 2014 with no concrete recommendations. I think that there would be differences between plot sizes in the US vs Europe, given the prevalence of much larger monocultures in the US (e.g., soybean, cotton, corn). Obviously, the foraging range of the target bee species is a driver of plot size. Given the large foraging range of honeybees, it is important not just to focus on the size of the treated field, but the representativeness of the surrounding landscape with respect to "typical growing regions." I would expect the same size plot immersed in a region with lots of attractive (alternative) forage would result in very different exposure of bees compared to one with limited alternative forage.

   One of the biggest concerns with full field studies of honeybees in the US is the perception of having a high likelihood of a Type II error (false negative) because field size/location do not capture high end exposures. One notable exception might be with almonds in California, where little else is in bloom during the time almonds bloom and large acreage exists for this crop (>1 million). Ultimately, a careful GIS based analysis of hypothetical honeybee forage areas over space and time might inform the size and location of full field studies.

9. How many replicates of independent sites do you consider appropriate? In the US, the minimum is 3, from different growing regions, but as discussed previously, this is not supported by 'hard' data.
10. What is the minimum number of hives/colonies/nests for each plot in semi-field studies and each site in field studies? For tunnel studies, this has been defined in guidance with honeybees. For large scale (semi-field) colony feeding studies, typically 7-8 honeybee colonies / site (reflecting different treatments) are required—with sites being separated by 1 mile or more. For full field studies, I am not aware of specifications of the minimum number of hives for any bee species. Obviously, for full field studies, one does not want to exceed the 'carrying capacity' of the location in terms of forage resources for the colonies. I don't have knowledge of how to determine this, but I expect it could be approximated based on the energetic requirements of a colony in relation to available forage resources (though the latter could be difficult to define). Commercial beekeepers might also have insights to this carrying capacity question, though they often use supplemental feeding which can inflate the number of colonies they keep at a given site.
11. What is the appropriate hive/colony/nest set up and housing requirements for HB, BB and SB? What are the most important local environmental conditions to be consider for these studies? I do not have sufficient knowledge to specifically address this question for BB and SB, but clearly, ensuring that critical environmental factors are met (temperature, forage resources, timing of nest initiation, disease/predator prevention, etc.) would apply. For HB, experience has shown that facing hives in different directions and with different coloration/patterns helps avoid cross foraging of colonies. Ensuring that hives are initiated in time for spring nectar flows is critical, along with sound beekeeping practices to prevent Varroa and other common pests/pathogens.
12. What are, on average, the foraging flight distances you would consider in field residue studies with HB, SB and BB? What could be the minimum distance between fields that would make them independent (i.e. no exchange of bees)? For semi-field colony feeding studies with HB, 1 mile (1.6 km) has been used as a minimum distance among sites. The concern in these CFS studies, however, is not cross foraging on a given field but rather but obtaining a sufficiently diverse foraging landscape is represented by each site. For field residue studies conducted in the US, sites are normally very far apart (hundreds of miles) to capture different climate, hydrologic, agronomic and landscape differences. If one were want to have multiple sites in the same region/landscape and use free foraging honeybees as part of the exposure metric (e.g., to gage variability in fidelity to treated fields, landscape dilution), then placing hives at least 5 miles apart would likely suffice given the maximum forage

distance often cited for honeybees. For BB, I am not aware of minimum distances being defined, but BB foraging distances are said to be typically within 1.5 km from the colony (e.g., Gradish et al 2019). For solitary bees, foraging distance is species specific (*e.g.*, mason bees are reported to forage within 300 m from the nest).

13. Which are the crops you preferably use and why? What are the advantages and disadvantages of the most commonly used crops (OSR, Phacelia, sunflower, apple,…?)? In your experience, do these crops yield high residue levels compared to other crops? Are they generally good surrogate crops? Is it possible to elaborate extrapolation rules (e.g. crop groups definition within which residue levels are expected to be similar) in case surrogate crops are used for the other crops that need to be evaluated in the regulatory context (e.g. the crop under evaluation has a limited ability to produce sufficient amounts of pollen and nectar for residue analysis)?
In the US, the selection of crops for residue studies must reflect the bee-attractive crops to which they are applied. The more crops to which the pesticide is applied, the greater number of residues studies is required. There is no pre-defined "surrogate" crop. To my knowledge, the evidence and analyse supporting the use of a surrogate crops to represent many other crops is weak, particularly for systemic pesticides—given the many different factors that govern the expression of residues in pollen and nectar. For non-systemic pesticides, however, the identification of a surrogate (e.g., worst case) crop might be more likely, since factors affecting uptake and translocation are not relevant. Here, flower physiology (e.g., physical availability and orientation of stamen & nectaries) in relation to pesticide spray would likely be an important factor to consider, as well as duration of bloom for a given blossom.

For systemic pesticides, most of my experience comes from analysis of residue data for the neonicotinoids and sulfoxaflor (See USEPA 2020a,b). Therefore, the conclusions from this analysis may not be applicable to other types of pesticides. That being said, I have found that crop-to-crop differences in application rate-normalized residues (RUD) measured at the same site and time often exceed 10X and occasionally 1000X when measured at different sites. Importantly, the impact of crop on residues differed among sites and among matrices in unpredictable ways. Generally speaking, crop-to-crop differences in RUDs within a crop grouping (e.g., citrus, stone fruit, etc) were similar in magnitude to site-to-site differences. Some notable exceptions exist, with soybean having very low residues for neonicotinoids even with foliar applications. Furthermore, we found that grapes had high residues in pollen compared to other berry crops. Method of application matters, with seed treatment applications resulting in low residues overall of the neonicotinoids

(usually < 10 ppb) and thus, crop-specific differences in residues were much less apparent.

I think that more curation and analysis of residue data needs to be done in order to fully address the grouping and representativeness of crops for residue studies. In order to remove the confounding influence of site on residue levels, such studies will likely need to be conducted on multiple crops at a given site or even under semi-controlled (greenhouse/shade house) conditions.

14. Do you exclude the presence of other competing crops/plants in the surrounding area in order to ensure exclusive foraging on the treated crop? If this is not the case, what is the maximum % area of alternative food in the foraging area you would accept, i.e. what you consider as normal and could still be representative? For USEPA risk assessment, we either use resides collected directly from the plant or from bees in tents on the treated crop. We presently do not apply a "landscape dilution factor" and therefore, do not consider residues from free-foraging bees for risk assessment purposes in part due to uncertainty in what constitutes "typical" variation in such landscape dilution factors. Such dilution factors will vary not only by site, but also over time. I believe much more data on honeybee forage fidelity is needed in order to address this question.
15. Which land use/cover types are considered as alternative forage areas? What methods are you aware that is appropriate for mapping alternative forage areas and resources within the bees' foraging range? I have limited expertise in GIS/land use data. With respect to honeybees, which forage on a wide variety of plants, my understanding is that typical GIS data layers for land cover may be to coarse to adequately delineate forage areas. Honeybees are highly opportunistic foragers and hives are successfully reared even in urban environments. Furthermore, mere presence of an attractive crop does mean the presence of forage, since bloom is required. We also lack sufficient knowledge to determine the importance of non-floral resources (e.g., extrafloral nectar) as resources when plants are not in bloom. Based on my very limited understanding of current GIS land cover data, I think 'real time' reconnaissance may be required to define alternative forage at a given site, possibly using drone technology.
16. In your experience, how feasible is to identify landscapes with land uses/covers that enable foraging to a higher extent on the focal field? I have no experience in this area.

Sampling methods

17. What are the most preferred sampling methods in the practice for collecting pollen and nectar and why (manual collection from flowers vs pollinators collection and for which matrix)? I have no direct

experience in conducting pollen and nectar sampling. However, for risk assessment purposes, I prefer to use plant-collected (e.g., capillary tube) and bee-collected sampling methods, where bees are tented. In general, I prefer bee-collected samples over plant-collected samples when both are available because I believe bees are better at integrating spatial differences in residue levels among plants and blossoms compared to hand techniques. I believe it is also more representative of what is being brought back to the hive.

For many crops, hand collection is not practical and bees must be used. One thing of concern is desiccation of pollen and how this affects residue concentrations that are expressed on a fresh weight basis. Hand sampling methods, particularly on day 0, can also result in contamination of sample due to transfer of residues remaining on petals.

18. Do you think the sampling technique (of the same matrix) can affect the residue levels estimation? Yes. For sulfoxaflor, we saw that residues in cotton pollen and nectar collected from plants were greater than those collected by tented bees and in turn, bee-collected residues were greater than those collected from tented hives. Hive samples can be problematic because it may not be known how long matrices have been deposited in the hive prior to collection.
19. Do you recommend a minimum quantity of sample for the various matrices? What is it? I have no experience in this area but experts on the ICPPR residue study team do and this is being addressed.
20. What is an appropriate time period to collect samples in relation to the phenology of the flowering crop/model plant? If bees are used, do you think that the season/time of the year should inform the selection of the bee type? Could samples be pooled across hives/colonies/nests? The timing for collecting samples will depend on the objectives of the study and nature of the application method and timing. For foliar spray applications where peak residues are the objective, collecting samples when sufficient blossoms are available soonest after application is appropriate. If the objective includes an understanding of residue kinetics, then more sampling events are required. As mentioned previously, soil applications of systemic pesticides may require a longer sampling period to capture peak residues.

Other

21. Could palynology be used to confirm foraging on the focal crop? Yes, for pollen foraging and if the focal crop is relatively unique to the forage area. This, however, would not necessarily confirm foraging for nectar.

22. Which protocol is the most appropriate/what is the most appropriate method to determine the plant species origin of pollen in a quantitative way? Why? I have no experience in this area.
23. Are there methods to determine the plant species origin of nectar? In such case, which and with what accuracy? I have heard that it may be possible to conduct palynology on nectar, which may have pollen grains intermixed in it. However, I am uncertain if this can be used to track the source of nectar as the pollen grains present may have come from other locations/sources.
24. For contact exposure assessment, what is the best practice to sample bees? I have no experience in this area.